# Delivery of Nanogel Formulations with Antimicrobial Peptides for the Treatment of Mycobacteriosis

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*Mycobacterium tuberculosis* is the human pathogen that causes Tuberculosis (TB). In 2015, 10.4 million TB cases and 1.8 million deaths were reported, placing this disease alongside HIV/AIDS as the deadliest infectious diseases. Current treatments rely in the administration of a cocktail of four first-line antibiotics during 6 months and, in the worst case scenario, a long-lasting treatment (24 months) with second-line drugs. The overuse or misuse of antimicrobial agents decreases the success of treatments and increases emergence of Multi-drug resistant (MDR) strains. Therefore, the development of new strategies for TB therapy is urgently needed. In this scope, antimicrobial peptides (AMPs) arise as promising candidates for TB treatment since they present high spectrum of antimicrobial activity, high efficacy at low concentrations and low propensity for bacterial resistance. Nevertheless, the low capacity of AMPs to reach the infected site and the use of high concentrations to overcome this problem limits its clinical application - this can be circumvented using a drug delivery system [1].
Recently, in a versatile, easy and reproducible manner, we developed a promising delivery system by grafting a hydrophobic molecule to Hyaluronic acid (HA). The amphiphilic conjugate self-assembles in aqueous environment, allowing the entrapment of bioactive molecules. We showed that LLKKK18-loaded nanogels cause no BMMΦ death and no significant effect in terms of the number of apoptotic cells, regardless the treatment or whether the BMMΦ were or not infected with *M. avium*. Additionally, *in vitro* incubation of macrophages with LLKKK18-loaded nanogel reduced the intracellular levels of both *M. avium* and *M. tuberculosis*. Most importantly, the capacity of macrophages to internalize HA was confirmed by *in vivo* results obtained in a model of TB infection, where just a few drug administrations over a short period yielded a promising 1.2-log reduction of the microbial burden [2].
In future work we intend to optimize the nanogel formulations, by promoting the encapsulation of LLKKK18 or LLKKK18/antimicrobial drugs in the nanocarriers, and unravel the *in vitro* immunomodulatory and metabolic effect promoted by the formulations in different cell models of mice and human. Since thorough optimization of the formulations and process will be carried out, the *in vivo* efficacy of an aerosol delivery of the formulations to infected mice will be assessed using the inEXPOSE inhalation system during one month. The susceptibility of MDR mycobacteria strains, and the eventual resistance mechanisms, that arise upon long *in vitro* treatments with LLKKK18 will also be studied.

References

[1] Silva, J.P., et al., Antimicrobial peptides as novel anti-tuberculosis therapeutics, Biotechnol Adv, 34(5), 924–940, 2016.

[2] Silva, J.P., et al., Delivery of LLKKK18 loaded into self-assembling hyaluronic acid nanogels for tuberculosis treatment, J Control Release, 235, 112-124, 2016.